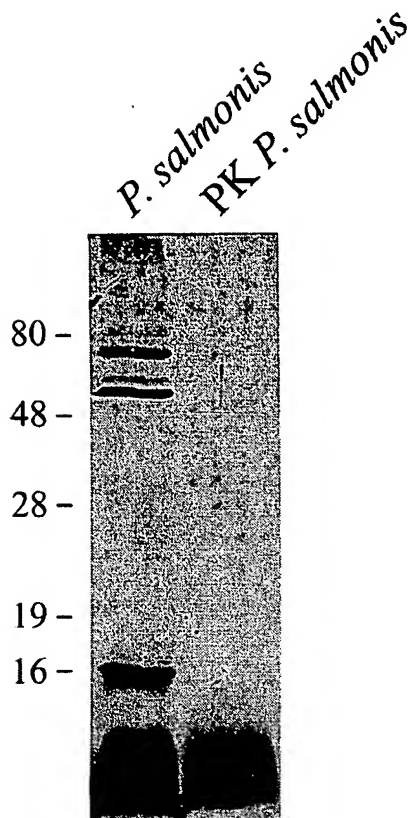


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Our Ref. No. 4616-67958  
In re application of: Kuzyk et al.  
For: VACCINES AND AGENTS FOR  
INDUCING IMMUNITY IN FISH  
AGAINST RICKETTSIAL DISEASES,  
AND ASSOCIATED PREVENTATIVE  
THERAPY  
Sheet 1 of 11

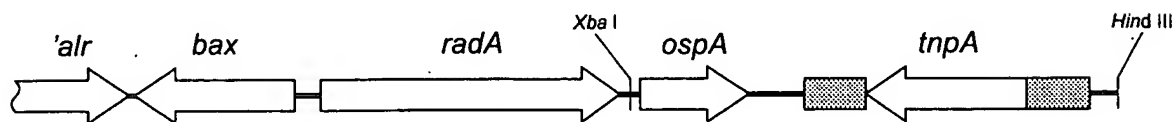
## FIGURE 1. WESTERN BLOT ANALYSIS OF *P. SALMONIS*



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## FIGURE 2

### A. ORF's in the region of the *ospA* gene from *P. salmonis*



### B. DNA sequence of *ospA* gene from *P. salmonis* (SEQ ID:1)

ATGAACAGAGGATGTTTGCAGGTAGTAGTCTAATTATTATCAGTGTGTTTTAGTTGGCTGTGCCCAGA  
ACTTTAGTCGTCAAGAAGTCGGAGCTGCGACTGGGGCTGTTGTTGGCGGTGTTGCTGGCCAGCTGTTTGG  
TAAAGGTAGTGGTCGAGTTGCAATGGCCATTGGTGGTGTCTGTTTTGGGTGGATTAATTGGTTCATAAATC  
GGTCAATCGATGGATCAGCAGGATAAAATAAAGCTAAACCAGAGTTTGGAAAAGGTAAAAAGCAGGGCAAG  
TGACACGTTGGCGTAATCCAGATACAGGCAATAGTTATAGTGTGAGCCAGTGCCTACTTACCAGCGTTA  
CAATAAGCAAGAGCGTCGCCAGCAATATTGTCGAGAATTCAGCAAAAGGCGATGATTGCAGGGCAGAAG  
CAAGAGATTTACGGCACTGCATGCCGGCAACCGGATGGTCTGTTGGCAAGTCATTTCAACAGAAAAA

### Amino acid sequence of OspA protein (SEQ ID:2)

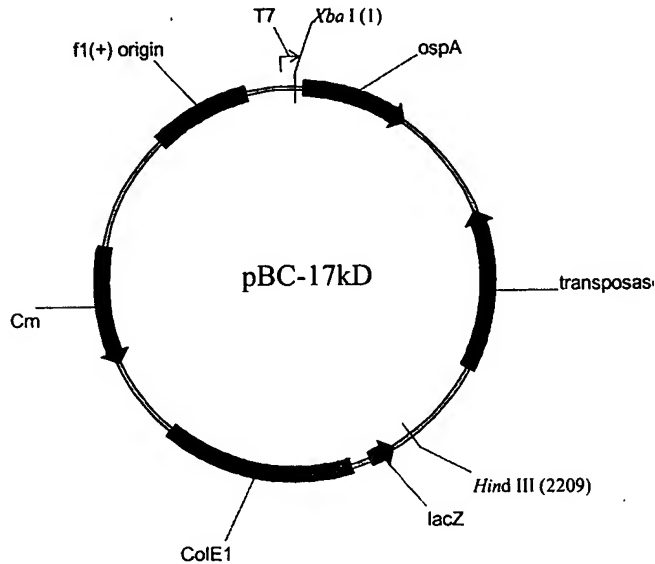
MNRGCLQGSSLI I I SVFLVGCQNFSRQEVGAATGAVVGGVAGQLFGKGSGRVAMAIGGAVLGGLIGSKI  
GQSMQDQDKIKLNSLEKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQYQYCREFOQKAMIAGQK  
QEIYGTACRQPDGRWQVISTEK

### C. Sequence alignment of the OspA proteins of *P. salmonis* and *R. prowazekii*

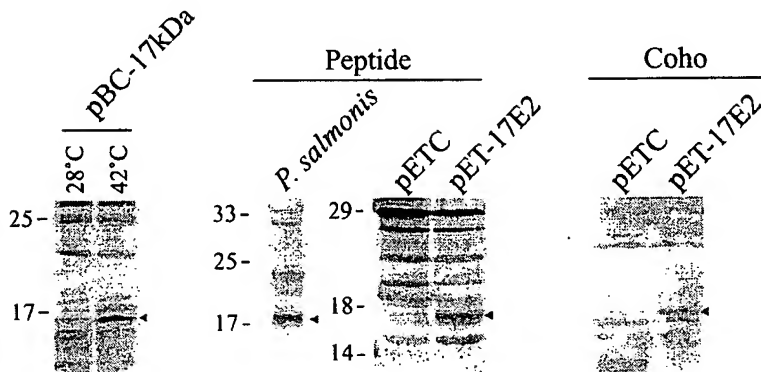
|                        |                     |                                 |              |                                  |
|------------------------|---------------------|---------------------------------|--------------|----------------------------------|
|                        | 10                  | 20                              | 30           | 40                               |
| <i>P. salmonis</i> :   | MNRGCLQGSSLI I I SV | FLVGC                           | QNF          | SRQEVGAATGAVVGGVAGQL             |
| <i>R. prowazekii</i> : | MKLLSKIMII I I AAS  | VLQ                             | NGSGM        | NKQGTGTLGGAGCILLCSQ              |
|                        | 10                  | 20                              | 30           | 40                               |
| <i>P. salmonis</i> :   | FCGKGS              | GRVAMAIGGAVLGGLIGSKI            | QSMQDQDKIKLN | QSL                              |
| <i>R. prowazekii</i> : | FGQCKG              | COLVGVGVGAILGAVLGGCIGASMDQDRLLE | LT           | SORALE                           |
|                        | 50                  | 60                              | 70           | 80                               |
| <i>P. salmonis</i> :   | FCGKGS              | GRVAMAIGGAVLGGLIGSKI            | QSMQDQDKIKLN | QSL                              |
| <i>R. prowazekii</i> : | FGQCKG              | COLVGVGVGAILGAVLGGCIGASMDQDRLLE | LT           | SORALE                           |
|                        | 50                  | 60                              | 70           | 80                               |
| <i>P. salmonis</i> :   | GVTRWRNP            | DGN                             | SYSE         | VPRTYQRYNKQERRQYQYCREFOQKAMIAGQK |
| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
| <i>P. salmonis</i> :   | GVTRWRNP            | DGN                             | SYSE         | VPRTYQRYNKQERRQYQYCREFOQKAMIAGQK |
| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
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| <i>P. salmonis</i> :   | GVTRWRNP            | DGN                             | SYSE         | VPRTYQRYNKQERRQYQYCREFOQKAMIAGQK |
| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
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|                        | 100                 | 110                             | 120          | 130                              |
| <i>P. salmonis</i> :   | GVTRWRNP            | DGN                             | SYSE         | VPRTYQRYNKQERRQYQYCREFOQKAMIAGQK |
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|                        | 100                 | 110                             | 120          | 130                              |
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| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
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|                        | 100                 | 110                             | 120          | 130                              |
| <i>P. salmonis</i> :   | GVTRWRNP            | DGN                             | SYSE         | VPRTYQRYNKQERRQYQYCREFOQKAMIAGQK |
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|                        | 100                 | 110                             | 120          | 130                              |
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|                        | 100                 | 110                             | 120          | 130                              |
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|                        | 100                 | 110                             | 120          | 130                              |
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| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
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| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
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| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
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|                        | 100                 | 110                             | 120          | 130                              |
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| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
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|                        | 100                 | 110                             | 120          | 130                              |
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| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
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|                        | 100                 | 110                             | 120          | 130                              |
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| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
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|                        | 100                 | 110                             | 120          | 130                              |
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|                        | 100                 | 110                             | 120          | 130                              |
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|                        | 100                 | 110                             | 120          | 130                              |
| <i>P. salmonis</i> :   | GVTRWRNP            | DGN                             | SYSE         | VPRTYQRYNKQERRQYQYCREFOQKAMIAGQK |
| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
| <i>P. salmonis</i> :   | GVTRWRNP            | DGN                             | SYSE         | VPRTYQRYNKQERRQYQYCREFOQKAMIAGQK |
| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
| <i>P. salmonis</i> :   | GVTRWRNP            | DGN                             | SYSE         | VPRTYQRYNKQERRQYQYCREFOQKAMIAGQK |
| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
| <i>P. salmonis</i> :   | GVTRWRNP            | DGN                             | SYSE         | VPRTYQRYNKQERRQYQYCREFOQKAMIAGQK |
| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
| <i>P. salmonis</i> :   | GVTRWRNP            | DGN                             | SYSE         | VPRTYQRYNKQERRQYQYCREFOQKAMIAGQK |
| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |
| <i>P. salmonis</i> :   | GVTRWRNP            | DGN                             | SYSE         | VPRTYQRYNKQERRQYQYCREFOQKAMIAGQK |
| <i>R. prowazekii</i> : | CSNIEPWRNP          | DGNHGYVTENKTYR                  | NSAGQYCRE    | YTC                              |
|                        | 100                 | 110                             | 120          | 130                              |

## FIGURE 3

### A. Map of plasmid pBC-17kDa encoding the *ospA* ORF.



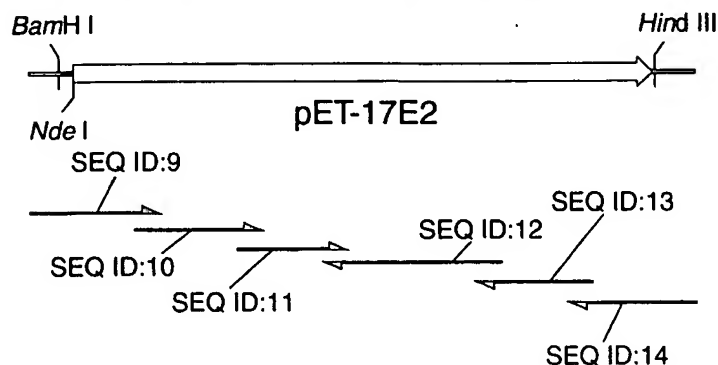
### B. Western blot analysis of OspA expression.



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**FIGURE 4.**

**A. Strategy for construction of the *E. coli* codon optimized *ospA* gene.**



**B. Oligonucleotide #1 (SEQ ID:9)**

CGCCAGGGTTTTCCCAGTCACGACGATCCGTCTCATATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATC  
 ATTATCTCTGTTTTCTGGTGGGTTGCGCCAGAACTTCAG

**Oligonucleotide #2 (SEQ ID:10)**

TGGGTTGCGCCAGAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGC  
 CGGCCAGCTGTTTCGGTAAAGGCTCTGGTTCGTGTGGCGATG

**Oligonucleotide #3 (SEQ ID:11)**

AAAGGCTCTGGTTCGTGTGGCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCG  
 GTCAGAGCATGGACCAGCAGGATA

**Oligonucleotide #4 (SEQ ID:12)**

GTTCCACAGAGTAGCTGTTACCGGTGTCCGATTACGCCAACGAGTAACCTGGCCGGCTTTCACTTTTTTC  
 CAGAGACTGGTTCAGTTTGATTTTATCCTGCTGGTCCATGCTCTGACC

**Oligonucleotide #5 (SEQ ID:13)**

GGTGCCGTAGATTTCTGTTTCTGACCTGCGATCATGGCTTTCTGCTGAAATTCGCGGCAGTACTGCTGA  
 CGGCGTTCCTGTTTGTGTAACGCTGGTAGGT

**Oligonucleotide #6 (SEQ ID:14)**

CGTCCTCTCGTCTGGTCCGAATTCAGATAAGCTTATTTTTCGGTGCTAATCACCTGCCAGCGGCCATCC  
 GGCTGACGGCACGCGGTGCCGTAGATTTCCTGTTTCTGAC

**C. DNA sequence of *E. coli* optimized *ospA* gene, 17e2 (SEQ ID:3)**

ATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCTGGTGGGTTGCGCCAGAACT  
 TCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGCCAGCTGTTTCGGTAA  
 AGGCTCTGGTTCGTGTGTTCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCGGT  
 CAGAGCATGGACCAGCAGGATAAAATCAAACCTGAACCACTCTCTGGAAGAAAGTGAAGCCGCCAGGTTA  
 CTCGTTGGCGTAATCCGGACACCGGTAACAGCTACTCTGTGGAACCGGTTGCGACCTACCAGCGTTACAA  
 CAAACAGGAACGCCGTGAGCAGTACTGCCCGCAATTTACAGCAGAAAGCCATGATCGCAGGTCAGAAACAG  
 GAAATCTACGGCACCGCGTGCCCTCAGCCGGATGGCCGTGGCAGGTGATTAGCACCGAAAAA

## FIGURE 5

### A. Amino acid sequence of optimized OspA protein, 17E2, (SEQ ID:4).

MRGCLQGSSLIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKSGSRVSMAGGAVLGGLIGSKIG  
QSMDDQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQQYCREFQQKAMIAGQKQ  
EIYGTACPQPDGRWQVISTEK

### B. DNA sequence of c17e2 *ospA* construct with N-terminal fusion partner (SEQ ID:5).

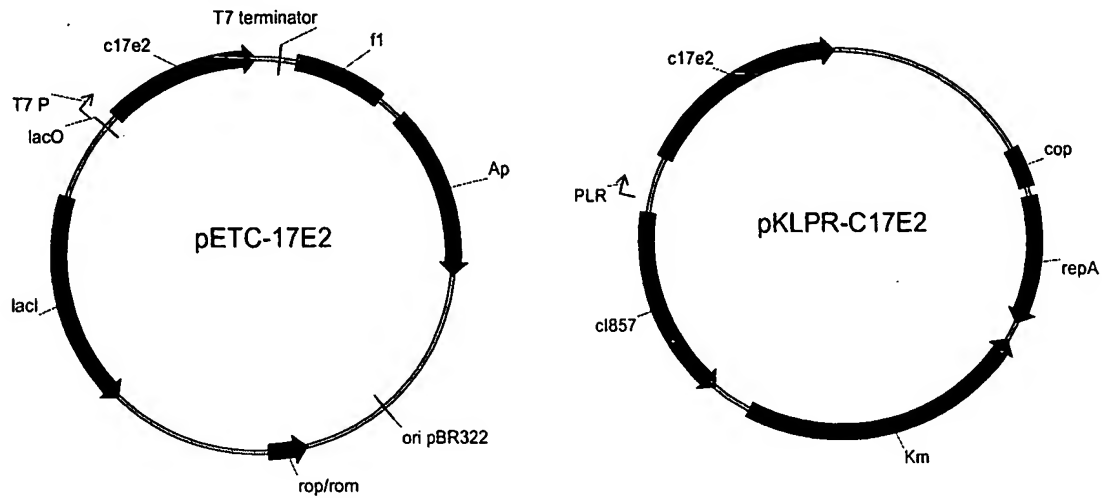
ATGTCAGTTGAATTCTACAACCTCTAACAAATCAGCACAAACAACTCAATTACACCAATAATCAAAATTA  
CTAACACATCTGACAGTGATTAAATTTAAATGACGTAAAAGTTAGATATTATTACACAAGTGATGGTAC  
ACAAGGACAAACTTTCTGGTGTGACCATGCTGGTGCAATTATTAGGAAATAGCTATGTTGATAACACTAGC  
AAAGTGACAGCAAACCTTCGTTAAAGAAACAGCAAGCCCAACATCAACCTATGATACATATCTGGATCCGT  
CTCATATGCGTGGTTGCCCTGCAGGGCAGCTCTCTGATCATTTATCTCTGTTTTCTGGTGGGTTGCGCCCA  
GAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGCCAGCTGTTC  
GGTAAAGGCTCTGGTCGTGTGTCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAA  
TCGGTCAGAGCATGGACCAGCAGGATAAAATCAAACCTGAACCACTCTCTGAAAAAGTGAAAGCCGGCCA  
GGTTACTCGTTGGCGTAATCCGGACACCGGTAAACAGTACTCTGTGGAACCGGTTTCGCACCTACCAGCGT  
TACAACAAACAGGAACGCCGTGAGCAGTACTGCCGCGAATTTTACGAGAAAGCCATGATCGCAGGTCAGA  
AACAGGAAATCTACGGCACCGCGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

### C. Amino acid sequence of C17E2 OspA construct with N-terminal fusion partner (SEQ ID:6).

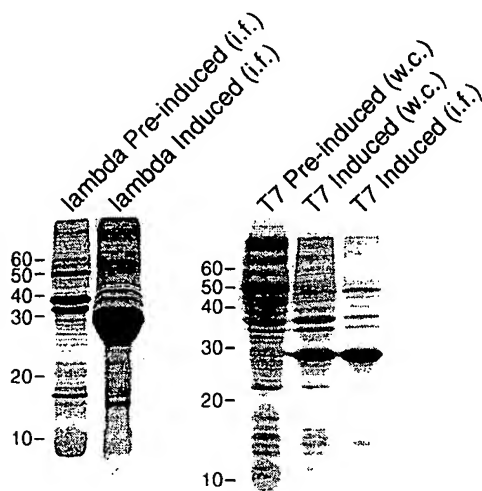
MSVEFYNSNKSQNTSITPIIKITNTSDSDLNLNDVKVRYYYTSDGTQGQTFWCDHAGALLGNSYVDNTS  
KVTANFVKETASPTSTYDITYLDPSHMRGCLQGSSLIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLF  
GKSGSRVSMAGGAVLGGLIGSKIGQSMDDQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQR  
YNKQERRQQYCREFQQKAMIAGQKQEIYGTACPQPDGRWQVISTEK

## FIGURE 6

### A. Expression vectors encoding the optimized *ospA* fusion constructs

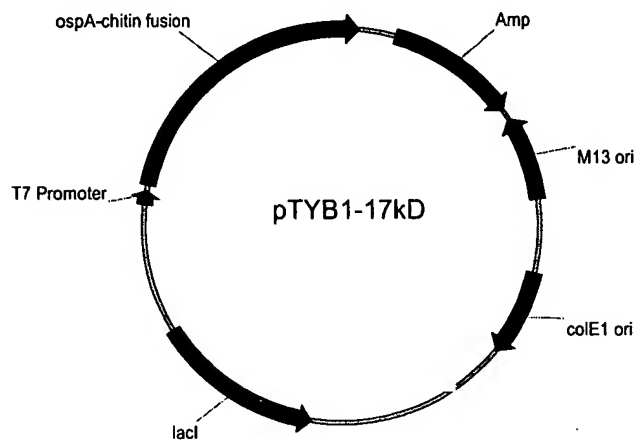


### B. SDS-PAGE analysis of C17E2 expression.



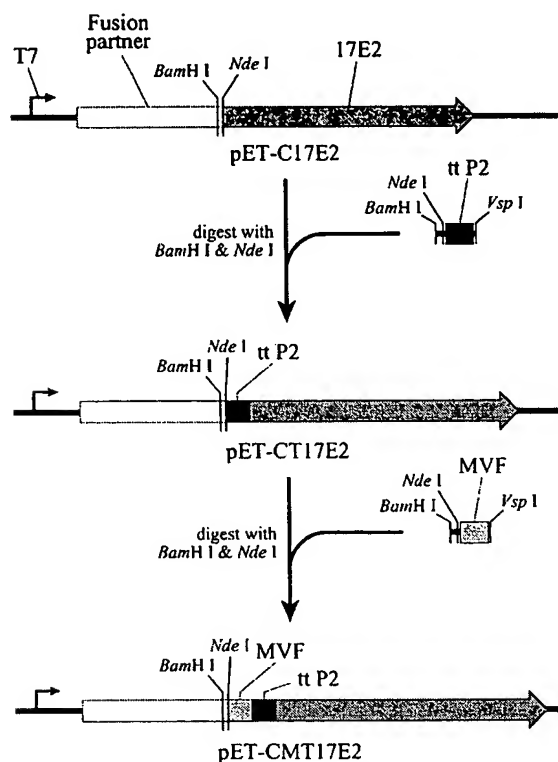
## FIGURE 7

**Map of the *ospA*-fusion construct encoding a C-terminal fusion partner under T7 promoter control.**



## FIGURE 8

### A. CLONING STRATEGY FOR OSP A TCE FUSION PROTEIN CONSTRUCTS.



### B. (a) Nucleotide sequence of the tt P2 oligonucleotide (SEQ ID:17)

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCAGTACATTAAAGCAAACCTCTAAATTCATC  
 GGTATTACCGAACTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

### (b) Nucleotide sequence of the MVF oligonucleotide (SEQ ID:18)

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCTGTCTGAAATCAAAGGTGTTATCGTTCAT  
 CGTCTGGAAGGCGTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

### (c) Amino acid sequence of the tt P2 TCE (SEQ ID:19)

QYIKANSKFIGITEL

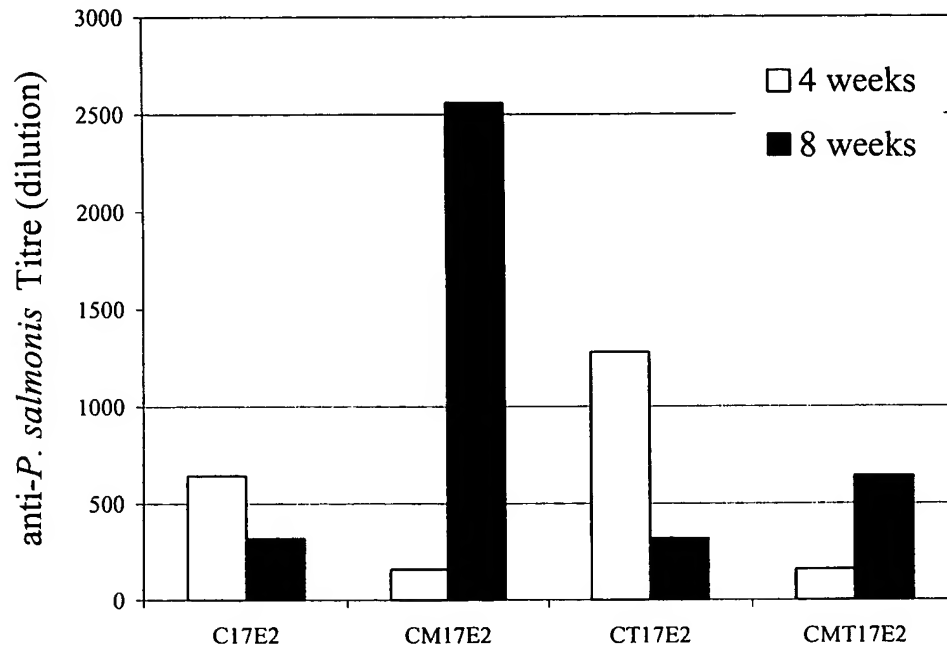
### (d) Amino acid sequence of the MVF TCE (SEQ ID:20)

LSEIKGVIVHRLEGV



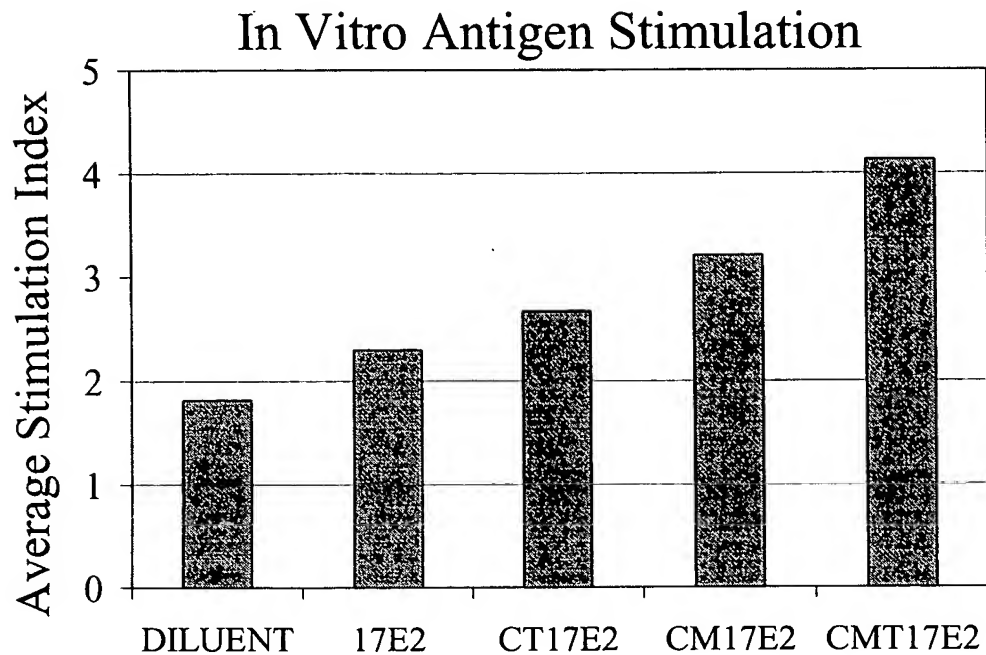
## FIGURE 9

**Coho salmon antibody titres against OspA-fusion protein  
candidate vaccines.**



## FIGURE 10

**Whole lymphocyte proliferative response to OspA-fusion proteins in Atlantic salmon.**



## FIGURE 11

### Vaccine trial in coho salmon of OspA fusion proteins.

